Bcl-2 and FKBP12 bind to IP_3 and ryanodine receptors at overlapping sites: the complexity of protein-protein interactions for channel regulation

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Abstract

The 12-kDa and 12.6-kDa FK506-binding proteins, FKBP12 and FKBP12.6, have been implicated in the binding to and the regulation of ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP_3Rs), both tetrameric intracellular Ca^{2+}-release channels. While the amino acid sequences responsible for FKBP12 binding to RyRs are conserved in IP_3Rs, FKBP12 binding to IP_3Rs has been questioned and could not be observed in various experimental models. Nevertheless, conservation of these residues in the different IP_3R isoforms and during evolution suggested that they could harbour an important regulatory site critical for IP_3R-channel function. Recently, it has become clear that in IP_3Rs, this site was targeted by B-cell lymphoma 2 (Bcl-2) via its BH4 domain, thereby dampening IP_3R-mediated Ca^{2+} flux and preventing pro-apoptotic Ca^{2+} signalling. Furthermore, *vice versa*, the presence of the corresponding site in RyRs implied that Bcl-2 proteins could associate with and regulate RyR channels. Recently, the existence of endogenous RyR/Bcl-2 complexes has been identified in primary hippocampal neurons. Like for IP_3Rs, binding of Bcl-2 to RyRs also involved its BH4 domain and suppressed RyR-mediated Ca^{2+} release. We therefore propose that the originally identified FKBP12-binding site in IP_3Rs is a region critical for controlling IP_3R-mediated Ca^{2+} flux by recruiting Bcl-2 rather than FKBP12. Although we hypothesize that anti-apoptotic Bcl-2 proteins, but not FKBP12, are the main physiological inhibitors of IP_3Rs, we cannot exclude that Bcl-2 could help engaging FKBP12 (or other FKBP isoforms) to the IP_3R, potentially via calcineurin.

Keywords:

Ca^{2+}, B-cell lymphoma 2, immunophilins, intracellular Ca^{2+}-release channels, protein complexes

Abbreviations:

B-cell lymphoma 2, Bcl-2; Bcl-2 homology, BH; 12.6-kDa FK506-binding protein, FKBP12.6; 12-kDa FK506-binding protein, FKBP12; 38-kDa FK506-binding protein, FKBP38; catecholaminergic polymorphic ventricular tachycardia, CPVT; cyclophilins, CP; cyclosporine A, CsA; dopamine- and cAMP-regulated phosphoprotein of 32 kDa, DARPP-32; inositol 1,4,5-trisphosphate receptors, IP_3R; protein kinase A, Jun N-terminal kinase, JNK; protein kinase A, PKA; protein phosphatase 1, PP1; ryanodine receptors, RyR

Introduction

Intracellular Ca^{2+} signalling regulates a variety of cellular processes [1]. A major organelle involved in the generation of these Ca^{2+} signals is the endoplasmic reticulum (ER), which not only is responsible for protein folding but also function as the main intracellular Ca^{2+} store [2]. The ER harbours two major protein families responsible for mediating Ca^{2+} release from the ER: the inositol 1,4,5-trisphosphate receptors (IP_3Rs [3]) and the ryanodine receptors (RyRs [4]). In short, three isoforms are known to exist for each intracellular Ca^{2+}-release channel, which show a differential distribution among tissues [5, 6]. In this way, IP_3Rs are ubiquitously expressed (at least one isoform in every cell), whereas high RyR expression is restricted to excitable and more specialized cells such as skeletal muscle (RyR1), cardiac muscle (RyR2), pancreas acinar cells (RyR1 and RyR2) and neurons (all isoforms). Both IP_3Rs and RyRs function as a tetrameric unit inserted via a C-terminal Ca^{2+}-permeable channel domain into the ER membrane. Due to their size (IP_3R>300 kDa and RyR>500kDa for their monomers), this organization results in a large cytosolic regulatory domain and a relatively small luminal domain. This cytosolic regulatory part of both channels is the target of several common regulatory mechanisms. Some of these regulators are cellular factors (Ca^{2+}, ATP, and Mg^{2+}), protein kinases and associated proteins [5, 6]. IP_3Rs and RyRs not only share functional and regulatory similarities, but also display structural similarities, e.g. at the level of their N-terminal domains and their distance to the transmembrane domains [7, 8].

The 12-kDa FK506-binding protein (FKBP12) and the 12.6-kDa FK506-binding protein (FKBP12.6) as critical regulators of RyR channels

An important regulatory protein associated with the RyRs is FKBP12 [9]. FKBPs together with cyclophilins (CPs) form the immunophilin family, characterized by its targeting by immunosuppressive drugs, including rapamycin and FK506 for FKBPs and cyclosporine A (CsA) for CPs [10, 11]. Complex formation of these drugs with FKBPs and CPs impacts phosphorylation events in the cell by targeting and inhibiting kinases and phosphatases. As such, the complex FK506/FKBP12 and the complex CsA/CP inhibit the Ca^{2+}/calmodulin-dependent phosphatase, calcineurin/PP2B [12], while the complex rapamycin/FKBP12 inhibits the mammalian target of rapamycin complex 1 [13, 14]. Besides this pharmacological signature, immunophilins display a *cis/trans* peptidylprolyl isomerase activity [15, 16].

FKBPs also form complexes with Ca^{2+} release channels, namely the RyRs [9]. Marks and co-workers reported the physiological and FK506-sensitive association of four FKBP12 molecules with the tetrameric RyR channel present in sarcoplasmic reticulum of skeletal muscle cells [17]. FKBP12-stripped RyR channels displayed increased Ca^{2+} flux and were sensitized to agonists like caffeine [18]. At the single-channel level, FKBP12 binding to RyR1 prevented the occurrence of sub-conductance

states, thereby stabilizing the channel in two conformations: (i) the closed state and (ii) the fully open state [19].

RyR/FKBP12-complex formation not only occurs in the context of the skeletal muscle, but also in cardiac muscle [20]. The immunophilin responsible for binding RyRs in the heart seemed a different FKBP isoform, namely FKBP12.6. However, this may be species dependent, since rat RyR2 mainly associated with FKBP12.6 while rabbit RyR2 exclusively associated with FKBP12 [21]. Single-channel analysis of cardiac RyR channels also revealed that rapamycin disrupted the binding of FKBP12.6 to the cardiac RyR protein [22]. FKBP12/FKBP12.6 not only promoted the coordinated Ca^{2+} flux among the four subunits of each RyR channel but also "coupled gating" [23]. This means that FKBP12/FKBP12.6 caused neighbouring or clustered RyR channels to function as a single unit that quasi-simultaneously open and close. Particularly, in the heart, these properties appear to be important to prevent aberrant and thus arrhythmic Ca^{2+} leakage during diastole and to facilitate proper Ca^{2+} flux during systole [24]. Besides immunosuppressive drugs, hyperphosphorylation of RyR2 at Ser2809 by protein kinase A (PKA) during heart failure or chronic elevated adrenergic stimulation has been reported to disrupt RyR2/FKBP12.6 complexes [25]. Yet, the position of the PKA-phosphorylated residue in RyR2 (Ser2808 *versus* Ser2030) and the relevance of PKA-mediated phosphorylation of RyR2 during heart failure have been questioned [26, 27], while PKA-mediated phosphorylation of RyR2 at Ser2808 did not dissociate FKBP12.6 from RyR2 [28]. Also, FKBP12.6-deficient mice have been reported to display exercise-induced sudden death due to aberrant RyR2 function [29]. In contrast to this, loss of FKBP12.6 has been reported to neither alter the functional properties of the cardiac RyR2 nor the tendency for spontaneous Ca^{2+}-release events to occur [30]. As a consequence, FKBP12.6-knockout mice were not more vulnerable to stress-induced ventricular arrhythmias [30]. Nevertheless, defective RyR2/FKBP12.6-complex formation has also been observed in RyR2 channels containing mutations that are associated with catecholaminergic polymorphic ventricular tachycardia (CPVT) [31]. Proper FKBP12.6 recruitment to and normal Ca^{2+} flux through these defective CPVT-mutated RyR2 channels could be pharmacologically restored using JTV519 [32], a benzothiazepine derivative which stabilizes RyR2/FKBP12.6 complexes. In addition to phosphorylation events, oxidizing reagents (like H_2O_2 and diamide) or pathological conditions associated with increased reactive oxygen species production have been implicated to negatively impact the binding of FKBP12.6 to oxidized RyR2 [33, 34]. Importantly, JTV519 could not restore this loss of FKBP12.6 binding to RyR2 under oxidizing conditions [33]. Also, other reports indicated that the anti-arrhythmic properties of JTV519 suppressed spontaneous Ca^{2+}-release events by CPVT-linked mutant RyR2 channels independently of FKBP12.6 [35]. Finally, it is important to note that different alternative models have been proposed for RyR2 modulation by FKBP12.6, including its ability to counteract the activation of RyR2 by FKBP12 [36].

Finally, also RyR3 channels form complexes with FKBP12 and FKPB12.6 that are sensitive to exposure to FK506 [37, 38]. FKBP12 and FKBP12.6 bound the RyR3 protein with similar efficiencies. The formation of these complexes appeared regulated by Ca^{2+} and Mg^{2+}, but not by cyclic ADP ribose. The binding of FKBP12 to RyR3 prevented the occurrence of spontaneous Ca^{2+}-release events (Ca^{2+} sparks), because overexpression of FKBP12 prominently reduced the number of spontaneous sparks in RyR3-expressing HEK293 cells [39].

The binding site of FKBP12/FKBP12.6 on RyR isoforms

Several groups have identified a potential FKBP12-binding site in the centre of the RyR-channel complex at a dipeptidylprolyl motif, in which the first amino acid is hydrophobic (Fig. 1). In RyR1, Val2461 preceding Pro2462 was identified as a key residue for the binding of FKBP12 [40]. Val2461-mutated RyR1 channels, defective in FKBP12 binding, displayed altered single-channel properties, including an increased open probability at low cytosolic [Ca^{2+}]. Furthermore, replacing Val2461 into Ile in the RyR1 sequence, resembling the corresponding residue in the RyR2 sequence, resulted in reduced FKBP12 binding and in increased FKBP12.6 binding. $RyR1^{Val2461Ile}$ expressed in HEK293 cells that contain endogenous FKBP12, but not FKBP12.6, displayed altered single-channel properties that could be corrected by adding FKBP12.6.

Also in RyR3, a central valine-proline motif was essential for FKBP12 binding (Fig. 1). Mutating Val2322 into Asp in mink RyR3 reduced FKBP12 binding, increased spontaneous Ca^{2+} puffs and increased sensitivity towards opening in response to caffeine [39].

In RyR2, the situation is more complex. Originally, it was proposed that the FKBP12.6-binding site is located in the central RyR2 domain (a.a. 2361-2496), which contains a Ile2427/Pro2428 pair (Fig. 1). In yeast two-hybrid assays, the binding of FKBP12.6 to the RyR2 fragment was disrupted by rapamycin [25]. Yet, the molecular determinants underlying FKBP12.6 binding to RyR2 have been debated [41]. For instance, an N-terminal fragment containing a.a. 305-1937 of RyR2 was found to be responsible for FKBP12.6 binding [42]. Using elegant cryo-EM analysis of GFP-inserted RyR2 channels and FRET-based studies and in agreement with previous reports of his group, Chen and co-workers convincingly showed that FKBP12.6 associates with the N-terminal portion of the RyR2 channels with a prominent role for two regions: a first region between a.a. 305 and 784 and a second region between a.a. 1815 and 1855. Interestingly, both regions are adjacent in the 3D RyR2 structure [43].

Very recently, the high-resolution structures of the RyR1 channel have been published [44-46]. From these structures, it appears that FKBP12 binding to RyR1 occurs *via* a site located in the N-terminal part of the RyR1 channel [45]. Hence, this analysis implies that not only RyR2 but probably also the other RyR isoforms harbour an FKBP12/FKBP12.6-binding site in their N-terminal region.

A potential FKBP12-binding site is conserved in the central regulatory domains of IP_3Rs

Besides RyRs, IP_3Rs, another class of intracellular Ca^{2+}-release channels, have been proposed by Snyder and co-workers to serve as targets for FKBP12, inhibiting the channel [47] (Fig. 2, model A). FKBP12 co-purified with IP_3Rs from rat cerebellar membranes in an FK506-dependent manner. FKBP12-stripped IP_3R increased the basal Ca^{2+}-leak from microsomal preparations and sensitized IP_3Rs towards activation by IP_3. FKBP12 did not function by itself but rather served as an adaptor protein responsible for linking calcineurin to the IP_3R [48]. Disrupting the binding of FKBP12 to the IP_3R also resulted in the loss of calcineurin recruitment to the IP_3R and loss of calcineurin-dependent dephosphorylation of the IP_3R channel. Hence, it was proposed that Ca^{2+} release through IP_3Rs could locally activate calcineurin and dephosphorylate IP_3Rs, thereby reducing its Ca^{2+}-flux properties, creating a negative feedback loop that is controlled by Ca^{2+} [48]. In contrast, inhibition of calcineurin by FK506 will abolish this negative feedback loop, thereby favouring phosphorylation of IP_3R channels (e.g. via PKA-dependent mechanisms) and enhancing IP_3R function and IP_3-induced Ca^{2+} release. At the molecular level, an FKBP12-binding site was identified in the central, modulatory domain of the IP_3R1 channel using a yeast two-hybrid assay [49]. In this region (a.a. 1349-1460), Pro1401 was critical for interaction with FKBP12, because its mutation abolished FKBP12 binding, while other Pro residues in the fragment were not important. The authors proposed that Pro1401 and the preceding Leu1400 residue served as an FK506-mimicking structure. Interestingly, the proposed binding site was conserved among the three different IP_3R isoforms (Fig. 1) and correlated with the sequences identified in RyRs to act as an FKBP12-binding site.

However, in contrast to these reports by Snyder and co-workers, other teams, including our laboratory, could neither detect IP_3R/FKBP12-complex formation [38, 50] nor alterations in IP_3R-mediated Ca^{2+} fluxes in response to FKBP12 overexpression [51], purified FKBP12 addition [37] or FK506 treatment [37, 52]. In our team, we have employed different experimental approaches combining biochemical and functional techniques, but none of these assays indicated that IP_3Rs could form complexes with FKBP12 or could be regulated by these proteins [37, 38, 52], while the same approaches could confirm FKBP12 binding to and regulation of RyR isoforms [37, 38]. Importantly, the amino acid sequence of the IP_3R1 corresponding to the proposed FKBP12-binding site placed in the context of the full-length RyR3 was able to bind FKBP12, indicating that the primary IP_3R1 sequence displayed FKBP12-binding properties in the proper structural environment of the RyR3 [38]. As such, it was postulated that the higher order structural organization of the leucylprolyl dipeptide motif was essential to serve as an FKBP12-binding site. Secondary structure predictions suggested that the structural micro-environment of the leucylprolyl motif in the IP_3R was completely different than the one of the motif in the RyR, where it served as an "alpha-helical breaker" [38, 53].

For a detailed discussion regarding the controversial role of FKBP12 for regulating IP_3R channels, we would like to refer to a recent review [54]. In any case, while the regulation of RyR channels by FKBP12 proteins has been consistently observed by different labs, it is clear that IP_3Rs are very poor targets of FKBP12 or that the interaction between IP_3Rs and FKBP12, if any, is strongly dependent on the cellular context or cell types.

The B-cell lymphoma 2 (Bcl-2) protein targets the FKBP12-binding site on IP_3Rs and RyRs

Strikingly, while the binding of FKBP12 to IP_3Rs may be very weak or strongly context dependent, its proposed binding site seems anyway to be important, because i) the amino acid sequence stretch surrounding the leucylprolyl motif is conserved among the different IP_3R isoforms and during evolution and ii) the corresponding motifs and surrounding amino acid sequences are present in the RyR isoforms and are responsible for FKBP12 and/or FKBP12.6 binding. A number of years ago, in collaboration with the laboratory of Clark Distelhorst, we could identify in mouse IP_3R1 the binding site for the anti-apoptotic Bcl-2 protein [55]. Importantly, this region encompassed the leucylprolyl motif identified as a putative FKBP12-binding site [49] (Fig. 1).

The Bcl-2-protein family consists of both anti- and pro-apoptotic family members and exerts its function through the presence of one to four Bcl-2 homology (BH) domains [56]. Anti-apoptotic Bcl-2 family members usually contain four of these BH domains (Fig. 3). The BH1, 2 and 3 domains form a hydrophobic cleft, which scaffolds the BH3 domains of the pro-apoptotic Bcl-2-family members, thereby neutralizing their pro-apoptotic function [57]. The BH4 domain is essential for the anti-apoptotic function of Bcl-2, since Bcl-2ΔBH4 fails to act as an anti-apoptotic protein [58]. The BH4 domain of Bcl-2, which can interact with several proteins [59, 60], was essential and sufficient to bind to IP_3R1 by targeting a 20 amino acid region (a.a. 1389-1408) within its central, modulatory domain [59, 61] (Fig. 3). Binding of Bcl-2 or its BH4 domain to the IP_3R channel dampened Ca^{2+} flux through the channel and protected the cells against apoptosis by suppressing excessive Ca^{2+} transfer from the ER to the mitochondria [59, 61]. Consistent with the fact that the amino acid stretch responsible for Bcl-2 recruitment to IP_3R1 is conserved among the various IP_3R isoforms (Fig. 1), the purified central, modulatory domains of IP_3R2 and IP_3R3 were also able to interact with full-length Bcl-2 as well as with its isolated BH4 domain region [62]. Interestingly, the functional implications of Bcl-2 binding to IP_3R resemble the ones originally described for FKBP12 binding to IP_3R, namely causing an inhibition of IP_3R-mediated Ca^{2+} flux.

In addition to this, it also became recently clear that in T cells and B-cell cancer cell models Bcl-2 not only binds directly to IP_3Rs but also serves as a docking protein responsible for the recruitment of calcineurin and dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), an inhibitor of protein phosphatase 1 (PP1) [63, 64] (Fig. 2, model B). DARPP-32 is activated by PKA and

deactivated by calcineurin. The formation of a complex between the IP_3R, Bcl-2, DARPP-32 and calcineurin allows a delicate control of IP_3-induced Ca^{2+} release. After T-cell stimulation IP_3-induced Ca^{2+} release is therefore first increased by the PKA-mediated IP_3R phosphorylation but subsequently repressed by the consecutive activation of calcineurin. This leads to the removal of DARPP-32-mediated inhibition of PP1, allowing PP1 to dephosphorylate and inactivate the IP_3R. As such, Bcl-2 has therefore two ways to limit IP_3R activity: i) via a direct inhibition in the central, modulatory domain, thereby negatively impacting the opening of the channel in response to IP_3 binding, and ii) via a regulatory mechanism involving calcineurin and PP1, thereby limiting phosphorylation of the IP_3R by PKA and preventing its hypersensitivity to IP_3. This recent study using T cells and B-cell cancer cells underpins previous observations obtained from using neuronal tissues [65-67]. These studies showed that calcineurin can bind Bcl-2, thereby linking calcineurin to IP_3Rs. IP_3Rs/calcineurin complexes could be observed in hippocampal lysates from Bcl-2 wild-type but not of Bcl-2-knockout mice.

Bcl-2 binding to RyRs

Driven by the striking resemblance of the identified Bcl-2-binding site on the IP_3R with an amino acid stretch present in the RyR sequence, we recently reported that Bcl-2 could also target RyRs [68]. Importantly, RyR/Bcl-2-protein complexes were identified in overexpression models and in rat hippocampal neurons [68]. It also became clear that Bcl-2 directly bound to RyRs via the same molecular determinant that involved interaction with IP_3Rs: i) the central, modulatory domain of all three RyR isoforms was able to bind full-length Bcl-2; and ii) the BH4 domain of Bcl-2 was sufficient to bind purified RyR fragments (from all three RyR isoforms) (Fig. 3). Also, at the functional level, the impact of Bcl-2 on RyR function resembled its effect on IP_3Rs, i.e. limiting Ca^{2+} release mediated by RyRs. In any case, it is clear that the RyR region corresponding to the Bcl-2-binding site in IP_3R (i.e. 2448-2469 in rabbit RyR1; 2415-2436 in rabbit RyR2; 2309-2330 in mink RyR3) overlaps with the previously identified dipeptidylprolyl motif serving as the FKBP12/FKBP12.6 binding site (Fig. 1). Hence, physiological complex formation between RyRs and Bcl-2 likely will depend on i) the relative expression/abundance of FKBP12 (or FKBP12.6) *versus* Bcl-2 in cell types and tissues, and ii) the affinity of FKBP12 (or FKBP12.6) and of Bcl-2 for binding to RyRs.

Bcl-2 proteins as targets of immunophilins

Clearly, Bcl-2 and its family members display functions that are unrelated to the regulation of apoptosis [69, 70]. Besides Bcl-2 proteins targeting and regulating IP_3Rs and RyRs via a putative or real FKBP12-binding site, Bcl-2 might also recruit immunophilins to IP_3Rs and RyRs, because Bcl-2 can form complexes with FKBP38, another immunophilin-family member [71, 72]. FKBP38 may

target Bcl-2 to the mitochondria and inhibit apoptosis [72], although other reports have indicated that FKBP38 binding to Bcl-2 rather promotes apoptosis [73]. Interestingly, FKBP38 peptidylprolyl isomerase activity critically depends on complex formation with Ca^{2+}/calmodulin [73]. It has been proposed that only the "active" Ca^{2+}/calmodulin/FKBP38 complex can bind Bcl-2 [73], which is consistent with findings that a charge-sensitive loop within the catalytic domain of FKBP38 is responsible for Bcl-2 binding [74]. This provides an interesting link between Ca^{2+} signalling and Bcl-2-protein function. FKBP38 interacts with Bcl-2 via its flexible loop (Fig. 3) and enhances its anti-apoptotic properties at the level of the mitochondria, potentially by stabilizing its structure, improving its folding and/or preventing its degradation by proteolytic enzymes like caspase 3 [75, 76]. In addition, FKBP38 appears to counteract Jun N-terminal kinase (JNK)-dependent phosphorylation of Bcl-2, which is implicated in neutralizing its anti-apoptotic function [76]. *Vice versa*, JNK-dependent phosphorylation of Bcl-2 hampered complex formation with FKBP38 [76]. These findings indicate that Bcl-2 proteins can interact with immunophilins and thus may recruit immunophilins to other Bcl-2 targets, like IP_3Rs (Fig. 2, model C). Of note, it remains to be elucidated whether Bcl-2 can form complexes with FKBP12. In any case, the observed interaction of FKBP12 proteins with IP_3Rs in some cellular models and conditions but not in others might therefore depend on the availability of Bcl-2, its presence in IP_3R-protein complexes, and the nature of the other proteins in the complex (as e.g. calcineurin and DARPP-32). Indirect binding of FKBP12 to IP_3Rs may thus occur via the Bcl-2/calcineurin complex (Fig. 2, model D). This could explain results from previous studies showing that (bacterially expressed and purified) FKBP12 could not directly bind to IP_3Rs. In addition, the isolation of intact ternary (IP_3R/Bcl-2/FKBP12) or of quaternary (IP_3R/Bcl-2/calcineurin/FKBP12) complexes may be very challenging and is likely strongly dependent on the experimental conditions used (e.g. cell types, detergents, solubilisation steps, etc…) and on the endogenous activity of the key players in the complex (e.g. active versus non-active calcineurin). This may in part underlie the controversy about IP_3R regulation by FKBP12 proteins [54].

Conclusions

RyRs have been identified as important targets of FKBP12 and FKBP12.6, members of the immunophilin family. A central dipeptidylprolyl motif was identified as the binding site for these proteins. This motif was embedded in a region that not only is conserved among all three RyR isoforms but also among the different IP_3R isoforms. As a consequence, also IP_3R channels were proposed to be targeted and regulated by FKBP12 by serving as an adaptor protein for calcineurin, leading to IP_3Rs dephosphorylation and thus limiting its activity. Strikingly, while the sequence appeared to be strongly conserved in IP_3Rs, its relevance for binding FKBP12 was subsequently questioned. Recent work however can explain the conservation of this site between the different IP_3R isoforms, revealing an important role in recruiting Bcl-2. Binding of Bcl-2 to IP_3Rs dampened its

Ca^{2+}-flux properties via a direct interaction as well as via the recruitment of calcineurin and DARPP-32. Thus, we hypothesize that Bcl-2 actually fulfils the role originally attributed to FKBP12 in the regulation of IP_3R channels. Importantly, since the site is shared among IP_3Rs and RyRs, Bcl-2 has been shown to interact with RyRs and to inhibit their Ca^{2+}-release activity. Hence, FKBP12 and Bcl-2 proteins may both serve as important regulatory proteins controlling IP_3R and RyR function. Yet, while the role of FKBP12 proteins may be limited to RyRs, Bcl-2 proteins may impact both IP_3Rs and RyRs. In addition to this, Bcl-2 proteins may serve as general adaptors, recruiting calcineurin/DARPP-32 and/or immunophilins to IP_3Rs and potentially RyRs. This definitely adds a further level of complexity to the fine-tuning of IP_3Rs and RyRs by immunophilins and Bcl-2.

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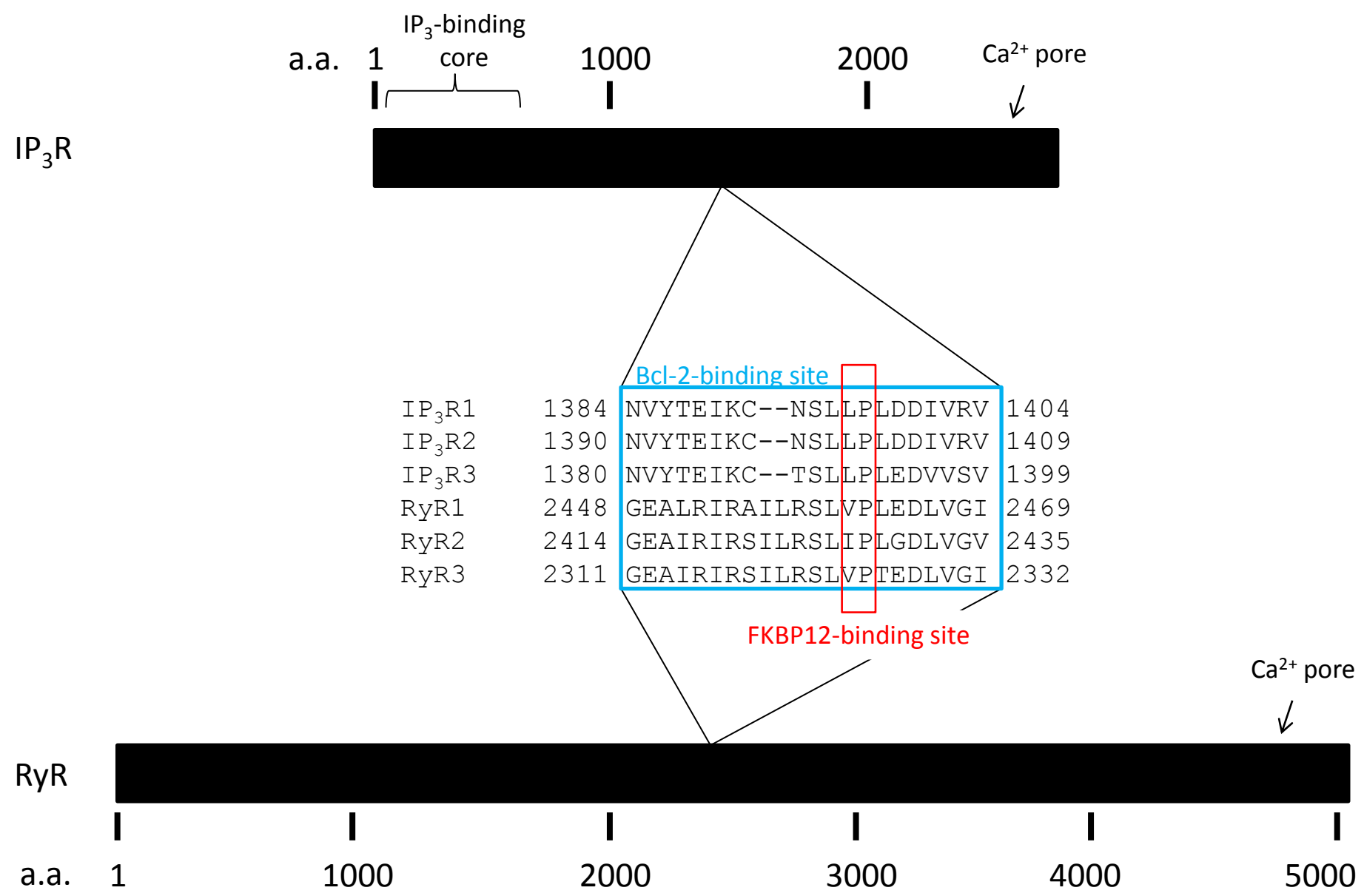
Figure legends

Figure 1. The proposed FKBP12- and Bcl-2-binding sites in the different human IP_3R and RyR isoforms. The conserved X-Pro motif (X=Leu, Ile or Val) proposed as the FKBP12 (or FKBP12.6)-binding site (red) and the conserved stretch of 20 amino acids identified as the Bcl-2-binding site (blue) in the central, modulatory domain of the different IP_3R and RyR isoforms have been indicated.

Figure 2. Possible models for the regulation of IP_3R channels by FKBP12 and Bcl-2 proteins, including their role as linker proteins recruiting calcineurin. Model A is based on the work of Snyder and co-workers [47] proposing that FKBP12 binds and inhibits IP_3Rs and link calcineurin to IP_3Rs. Calcineurin-mediated dephosphorylation of IP_3Rs would participate in suppressing IP_3R activity. Model B is based on the work of Distelhorst and co-workers [63] showing that Bcl-2 binds and inhibits IP_3Rs, thereby recruiting calcineurin and DARPP-32, a PKA-regulated inhibitor of protein phosphatase 1 (PP1). Model C is a hypothetical tripartite complex in which, in analogy with Bcl-2's ability to bind FKBP38, it is proposed that Bcl-2 might bind FKBP12 and link it to IP_3Rs. Model D is a hypothetical complex based on model B but in which additionally FKBP12 participate in the complex by binding to calcineurin.

Figure 3. Linear representation of the Bcl-2 structure and its four BH domains. The anti-apoptotic function of Bcl-2 is executed by the hydrophobic cleft formed by the BH3, BH1 and BH2 domains, scaffolding and neutralizing pro-apoptotic BH3-only proteins and multi-domain Bax/Bak proteins. The additional presence of the BH4 domain is needed for Bcl-2's anti-apoptotic function. Via its BH4 domain, Bcl-2 interacts with different Ca^{2+}-transport systems, like the IP_3R and the RyR and with Ca^{2+}-dependent enzymes like calcineurin. Via its flexible loop, Bcl-2 binds the "active" Ca^{2+}/calmodulin/FKBP38 complex via a Ca^{2+}-dependent interaction. FKBP38 may prevent JNK-dependent phosphorylation and/or caspase-3-mediated cleavage of Bcl-2.


IP3-binding core
a.a.
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2000
Ca2+ pore
IP3R
Bcl-2-binding site
IP3R1 1384 NVYTEIKC--NSLLPLDDIVRV 1404
IP3R2 1390 NVYTEIKC--NSLLPLDDIVRV 1409
IP3R3 1380 NVYTEIKC--TSLLPLEDVVSV 1399
RyR1 2448 GEALRIRAILRSLVPLEDLVGI 2469
RyR2 2414 GEAIRIRSILRSLIPLGDLVGV 2435
RyR3 2311 GEAIRIRSILRSLVPTEDLVGI 2332
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Ca2+ pore
RyR
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1000
2000
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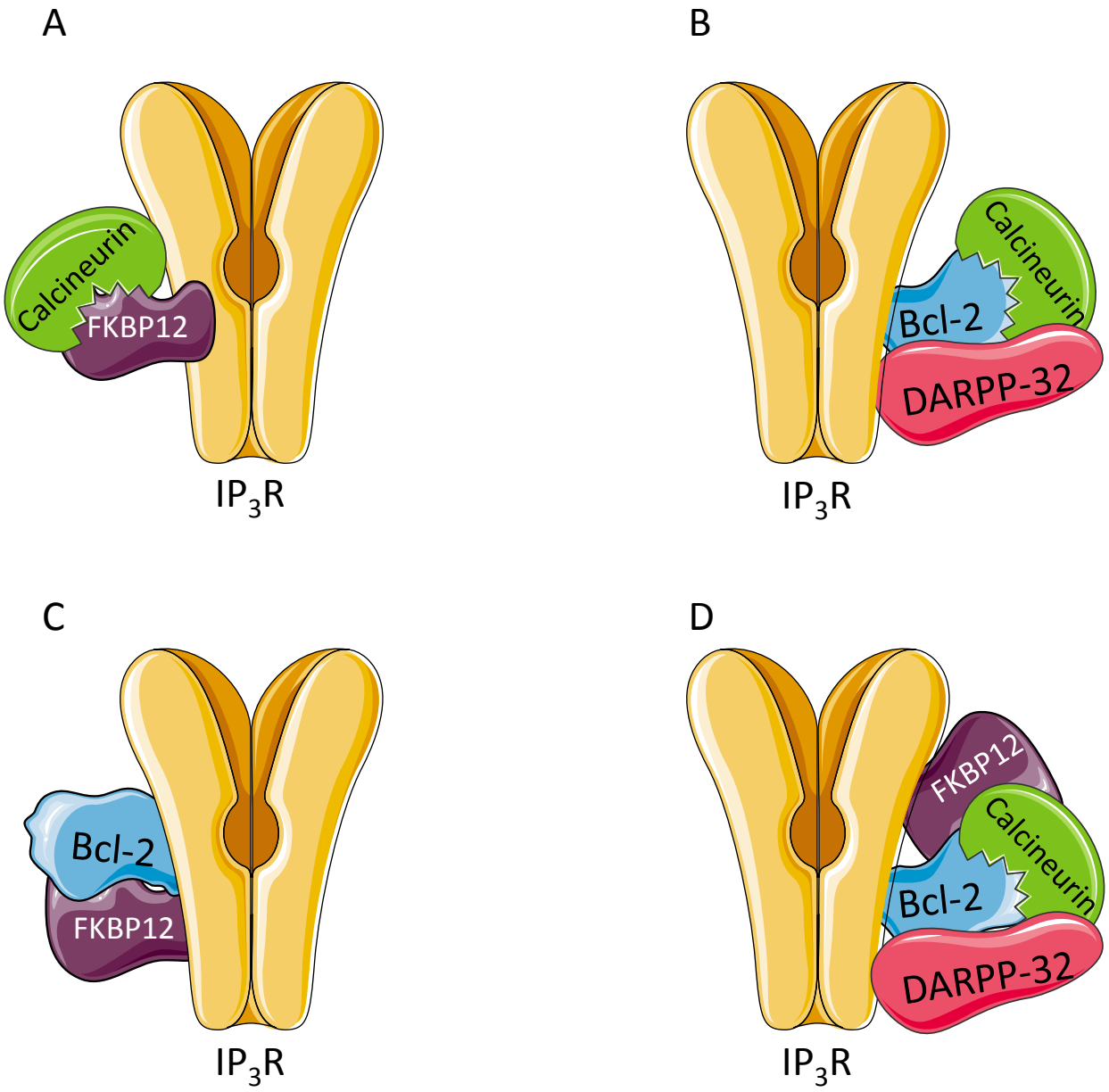
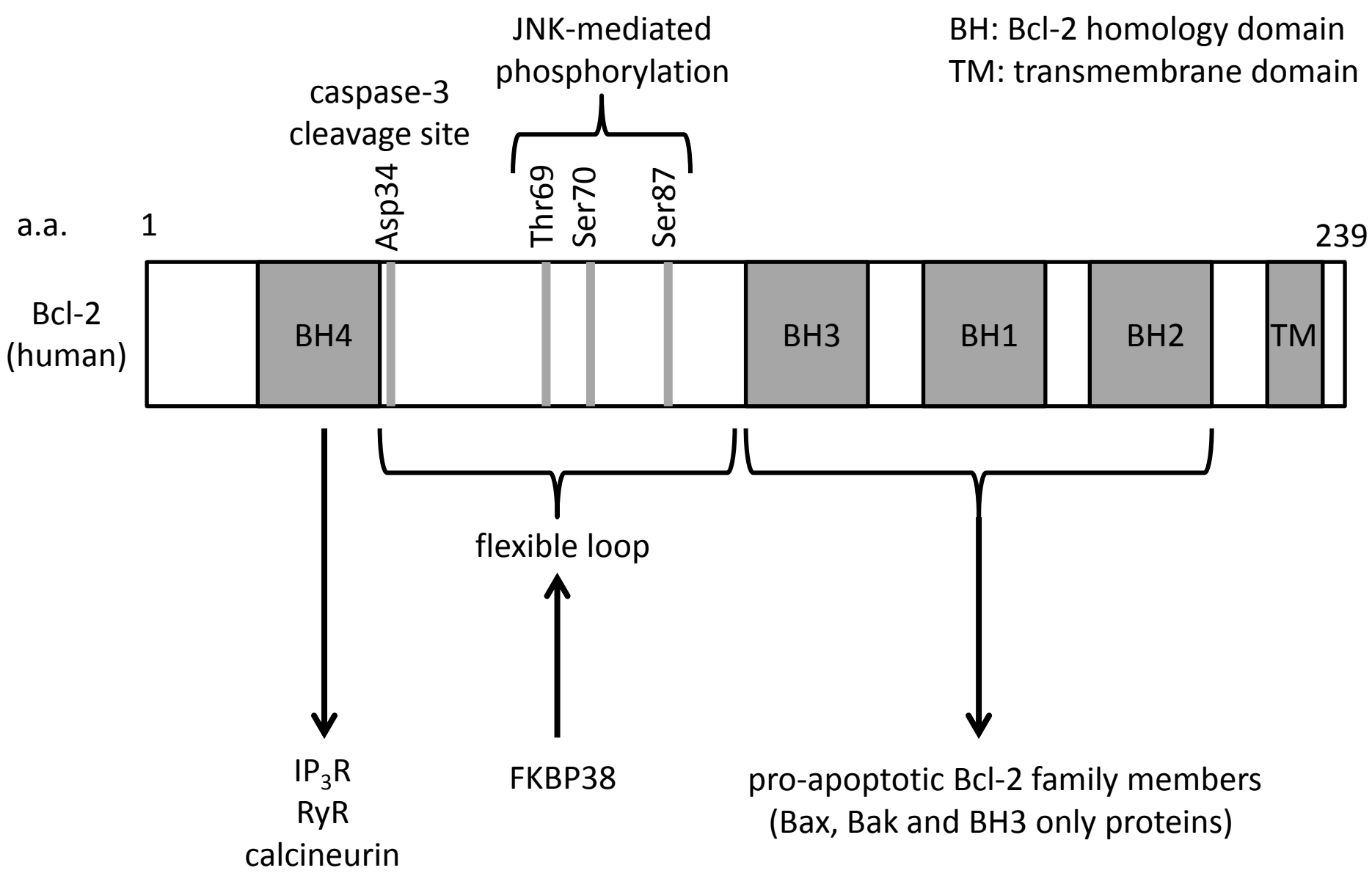

A
Calcineurin
FKBP12
IP3R
B
Calcineurin
Bcl-2
DARPP-32
IP3R
C
Bcl-2
FKBP12
IP3R
D
FKBP12
Calcineurin
Bcl-2
DARPP-32
IP3R


JNK-mediated
phosphorylation
BH: Bcl-2 homology domain
TM: transmembrane domain
caspase-3
cleavage site
Asp34
Thr69
Ser70
Ser87
a.a.
1
239
Bcl-2
(human)
BH4
BH3
BH1
BH2
TM
flexible loop
IP3R
RyR
calcineurin
FKBP38
pro-apoptotic Bcl-2 family members
(Bax, Bak and BH3 only proteins)